Global Journal of Animal Breeding and Genetics

ISSN: 2408-5502 Vol. 3 (3), pp. 117-124, March, 2015. © Global Science Research Journals
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Full Length Research Paper

Sequence variation in the cathepsin B (*CTSB*), L (*CTSL*), S (*CTSS*) and K (*CTSK*) genes in Ukrainian pig breeds

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Accepted 5 February, 2015

Abstract

Cathepsins are a family of peptidase enzymes which are involved in the regulation of economically important traits in pigs. The aims of this study were (i) to identify the breed-specific frequency of polymorphisms in the cathepsin B (*CTSB*), L (*CTSL*), S (*CTSS*), and K (*CTSK*) genes in seven Ukrainian pig breeds, and (ii) to compare the cathepsins polymorphism frequency between Ukrainian breeds, a commonly used European meat breed (Piétrain) and a representative fat breed (Meishan). The Ukrainian breeds used were: Ukrainian Large White, Ukrainian Large Black, Poltava Meat, Myrgorod, Ukrainian Steppe Spotted, Ukrainian Landrace and Ukrainian Steppe White. The study established breed-specific differences in the allele frequencies and informativity of polymorphisms in the *CTSB*, *CTSL*, *CTSS* and *CTSK* genes in Ukrainian breeds. The following were of particular interest: locus *CTSB* in Ukrainian Large Black breed, locus *CTSL* in Ukrainian Steppe Spotted, and loci *CTSS* and *CTSK* in Myrgorod breed. The segregation of SNPs in meat quality candidate genes demonstrating intermediate or high polymorphism levels in the Ukrainian breeds Myrgorod, Ukrainian Steppe Spotted and Ukrainian Large Black is of interest for future evaluation in association studies with the view of complementing selection indexes with molecular information.

Keywords: Pig, Ukrainian breeds, cathepsin genes, polymorphism frequency, genetics

INTRODUCTION

Tenderness is considered to be the most important meat quality trait from consumers' point of view (Miller et al., 2001). Intramuscular fat content and proteolysis during meat tenderisation process are the main determinants of tenderness. Cathepsins are a family of peptidase enzymes which are present mainly in the lysosomes of the skeletal muscles (Sentandreu et al., 2002). In the skeletal muscle, actin, myosin and associated proteins are the main targets of these peptidases and, therefore, they play a relevant role *post-mortem* in the process of conversion of muscles to meat (O'Halloran, 1997; Virgili et al., 1998).

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In relation to the pig, cathepsins are involved in defining economically important traits related to the quality of pork and pork products including meat texture, taste, softness and carcass lean content (Virgili et al., 1995; Garcia-Garrido et al., 2000); and have been linked to the yield and quality of dry-cured ham (Toldra et al., 1993; Ramos et al*.,* 2005). Polymorphisms in cathepsins S (*CTSS*), L (*CTSL*), F (*CTSF*), D (*CTSD)* and Z (*CTSZ*) have been related to pig performance traits such as average daily gain, feed/weight gain ratio, back fat thickness and weight of lean cuts (Russo et al., 2008; Fontanesi et al., 2010a; Speroni, 2010; Dvorakova et al., 2011; Fontanesi et al., 2012; Piorkowska et al., 2012). In heart myocytes, cathepsin S degrades cathepsin K (*CTSK*) which is responsible for obesity-associated cardiac hypertrophy and ischemia-induced neovascularisation (Hua et al., 2013; Jiang et al., 2014). *CTSK* has also recently been suggested as a new candidate gene for meat quality and fat content in pigs, and association between *CTSK* polymorphisms and backfat thickness has been reported (Fontanesi et al., 2010b).

Most studies on the cathepsin genes have been conducted on commonly used European breeds such as Italian Duroc, Large White, Landrace and Italian Large White, and breed-specific polymorphisms have been identified (Russo et al., 2002; Russo et al., 2003; Fontanesi et al., 2010a; Fontanesi et al., 2012). Relatively recently, these studies have expanded to Chinese native breeds and traditional Eastern-European pig breeds. Significant differences in *CTSD* genotype frequencies were observed between native Chinese breeds (Dahuabai and Erhualian) and Western breeds (Duroc and Landrace) (Mei et al., 2008). Information about breed-specific cathepsin polymorphisms can provide relevant data to build selection strategies for improving meat quality and pig performance traits.

Ukrainian traditional breeds are known for their high quality meat as well as for the ability to adapt effectively to difficult climate conditions (FAO, 2014). For example, the Ukrainian Steppe White is known for its tender and juicy meat, good reproductive performance and it is well adapted to hot dry climate. The Myrgorod breed is characterised by high content of intramuscular fat, tender meat, high water holding capacity, excellent flavor with high rate of weight gain. During the recent decades, the pig breeding programs in Ukraine increasingly include Piétrain and Meishan lines. Piétrain is a European breed with a high meat content but low in intramuscular fat, whilst Meishan, a Chinese breed, is known for high fat content. In order to ensure effective selection, it is important to have information about polymorphisms in genetic markers for meat quality traits in both, traditional Ukrainian breeds and in the breeds which have increasingly included in Ukrainian breeding programs.

In spite of high quality of meat and other advantages, there are scarce studies on the genetics of Ukrainian pig breeds. The use of Ukrainian breeds in European breeding programs could contribute to both, improving pork quality in Europe and economically sustain pig breeding industry in Ukraine. To progress in this direction, a better understanding of breed-specific biomarkers for meat quality traits in Ukrainian breeds is needed. The objectives of this study were: (i) to identify the breed-specific frequency of polymorphisms in the four cathepsin genes (*CTSB, CTSL, CTSS*, and *CTSK*) in seven Ukrainian breeds, and (ii) to compare the cathepsins polymorphism frequency between Ukrainian breeds, a commonly used European breed (as a representative meat breed) and a Chinese breed (as a representative fat breed).

MATERIAL AND METHODS

Animals and sample collection

The study was conducted on seven breed lines which were produced in Ukrainian pig breeding programs: Ukrainian Large White (n = 67), Ukrainian Landrace (n = 35), Ukrainian Large Black (n = 42), Ukrainian Steppe White (n = 10), Ukrainian Steppe Spotted (n = 20), Myrgorod (n = 20), and Poltava Meat (n = 20). The study also included Piétrain pigs (n = 9) as a European breed characterized by high muscularity and low fat content and Meishan pigs (n = 5), a Chinese breed with a very high fat content, as controls of extreme allelic frequencies for these genes. The animals were raised at the facilities of Ukrainian pig breeding companies which are under management of Institute of Pig Breeding and Agro-Industrial Production, National Academy Agricultural Sciences of Ukraine. The animals were fed a standard commercial diet (Poltava Feed Mill, Poltava, Ukraine). All the procedures related to rising of the animals and samples collection were conducted in accordance with the protocol approved by the Scientific Committee of the Institute of Pig Breeding, Poltava, Ukraine, and in accordance with the regulations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 1986. The protocol of this study was approved by the Institutional Animal Care and Use Committee, namely by the Scientific Methodological Committee of the Institute of Pig Breeding and Agro-Industrial Production, National Academy of Agricultural Sciences of Ukraine (Permit Number 2, 17.05.2011).

Samples of blood (1mL) were collected in the morning before feeding, mixed with 0.05 M EDTA in proportion 6 : 1 and stored for up to seven days at +4 °C until used for DNA isolation.

PCR Amplification

Genomic DNA was isolated from blood samples by sorbent met Hdd using Diatom™ DNA Prep 100 kit

Table 1: Primers and conditions for PCR amplification of cathepsin genes

Gene Name	Primer sequence (5'□ 3')	PCR size (bp)	Tm (ºC)	$MgCl_2$ (mM)
Cathepsin B	Fw: GTGGCCGGGTGGGTTTTA Rv: TCCTCCTGGTGCTGCTAATTCTGAC	139	55	2.
Cathepsin L	Fw: TCACTGCCGTGAAGAATCAG Rv: GCAGAGCTGTAATGGCAAGA	380	64	2.5
Cathepsin S	Fw: AGAGAGCCAGAGGTTGCTCA Rv: GCAGGCAGAGCAAGCTAAA	280	58	1.5
Cathepsin K	Fw: TTGGGCGATATGGTGAGTTGAG Rv: CATAAGAAAGGAACCAAGGCAAACA Probe-G: VIC-CAGCTCCT**G**GTCTATC-NFQ Probe-A: FAM-TCAGCTCCT**A**GTCTATC-NFQ	66	60	3.0

(Isogen, Moscow, Russia) with guanidyntiocyanate as a lysis reagent.

PCR for PCR-RFLP genotyping was carried out in a final volume of 20 µL that included 10 pM of each of the primers, 2.5 mM of each dNTP, 10-100 ng of genomic DNA and 1 unit of recombinant Taq DNA Polymerase (Fermentas, Vilnius, Lithuania). Concentration of $MgCl_2$ in the PCR reaction ranged from 1.0 to 2.5 mM as shown in Table 1.

Genotyping

CTSB, CTSL, and *CTSS* were genotyped using Restriction Fragment Length Polymorphism Analysis of PCR-Amplified Fragments (PCR-RFLP) technique as described by Russo et al. (2008) and Fontanesi et al. (2010a). The sequences of the primers are given in Table 1. *CTSB* was genotype on the SNP AJ315558, g.72 A>C. *CTSL* was genotyped on the SNP FN556187, g.143C>T, *CTSS* was genotyped on the SNP FN556188, g.171 G>A. For *CTSB* restriction analysis, 5 µl of PCR product was digested overnight at 37 ˚C with 3 units of *Msp*I restriction enzyme (Fermentas, Vilnius, Lithuania) in a final volume of 25 µL. *CTSL* PCR product was digested under the same conditions as for *CTSB* but in the presence of *Taq*I endonuclease (Fermentas, Vilnius, Lithuania) at 65ºC. The digests were separated in 2% agarose gel. Restriction analysis of *CTSS* amplification product was carried out by digestion with *Bse*NI endonuclease (Fermentas, Vilnius, Lithuania) at 65ºC and the digests were separated in 8% polyacrylamide gel.

CTSK was genotyped on the SNP FM209043, g.15G > A by TaqMan SNP Genotyping Assays (Life Technologies, USA) as described by Fontanesi et al. (2010b). The oligonucleotide probe sequences are given in Table 1.

Marker Data and Statistical Analysis

Allele frequencies, genotype frequencies, polymorphic information content (PIC) and levels of heterozygosity (observed heterozygosity, H_o and expected heterozygosity, $H_{e)}$ were calculated within each breed using the software and methodology described by Peakall (2006). H_o is heterozygosity for a single locus within a population where the number of heterozygotes was determined by direct count. H_e reflects genetic diversity within a population and was calculated per locus as 1 minus the sum of the squared allele frequencies.

For each SNP, significance of differences in allele frequencies between breeds was calculated by Fisher's F-test. Linkage disequilibrium between *CTSS* and *CTSK* makers was calculated using Haploview software (Barrett et al., 2005). Two estimations of linkage disequilibrium were used: D' (the deviation of the observed frequency of a haplotype from the expected (D), normalised to the frequency of the alleles in the two loci) and R^2 (the correlacion of D adjusted to the loci having different allele frequencies).

Phylogenetic Tree Analysis

The phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (Tamura et al., 2007) following the neighbor-joining method (Nei and Kumar, 2000) which is based on Edwards genetic distances. Data from the four cathepsins genes were used in the analysis.

RESULTS AND DISCUSSION

In the present study, the allelic distribution of polymorphisms in the four cathepsin genes were analysed in seven Ukrainian pig breeds. Regarding the *CTSB* locus, the study genotyped a g.72A>C SNP mutation in intron 6, which has been previously related to back fat thickness (Russo et al., 2002). This SNP segregated in all the Ukrainian populations analysed, with C always being the less frequent allele with a low minor

Table 2: Genotypes, allele frequencies and heterozygosity of cathepsin B (*CTSB*) g.72A>C polymorphism in different breeds of pigs

	Genotype frequency			Allele frequency				
Breed (n)	**g.72AA**	**g.72AC**	**g.72CC**	**g.72A**	**g.72C**	**H_o[a]**	**H_e[b]**	**PIC[c]**
Ukrainian Large White (67)	0.985	0.015	0.00	0.99[d]	0.01	0.015	0.015	0.020
Poltava Meat (20)	0.70	0.30	0.00	0.85[e]	0.15	0.300	0.255	0.222
Ukrainian Landrace (35)	0.97	0.03	0.00	0.99[d]	0.01	0.300	0.019	0.020
Ukrainian Steppe Spotted (20)	0.90	0.10	0.00	0.95[de]	0.05	0.100	0.095	0.090
Ukrainian Steppe White (10)	0.80	0.20	0.00	0.90[de]	0.10	0.200	0.180	0.164
Myrgorod (20)	0.85	0.15	0.00	0.93[de]	0.07	0.150	0.130	0.122
Ukrainian Large Black (42)	0.26	0.62	0.12	0.57[f]	0.43	0.620	0.490	0.370
Piétrain (9)	1.00	0.00	0.00	1.00	0.00	0.000	0.000	0.000
Meishan (5)	1.00	0.00	0.00	1.00	0.00	0.000	0.000	0.000

[a]Observed heterozygosity, [b] Expected heterozygosity, [c] Polymorphic information content,
Allele frequencies with different superscripts differ significantly,
$P < 0.05$ within Ukrainian pig breeds, (n) = number of animals

allele frequency (MAF) in most of the breeds in the range of 0.01 - 0.15. The only exception was Ukrainian Large Black breed, which had a MAF of 0.43 (Table 2). This might be related to the origin of this breed or to the duration of its selection in Ukraine. In contrast to the majority of Ukrainian breeds, the g. 72A>C mutation did not segregate in samples of Piétrain and Meishan pigs used in this study, both of which had the A allele fixed. The distribution of alleles are consistent with findings of Russo et al. (2008) that g.72A is the most frequent *CTSB* allele in Landrace, Piétrain and Meishan breeds with frequency values of 0.94, 0.95 and 1.00 respectively.

CTSB H_o, which is an indicator of genetic variability, ranged from 0.015 in Ukrainian Large White to 0.620 in Ukrainian Large Black. The H_e values for this locus did not differ significantly from H_o values in all the breeds investigated. However, it should be noted that in Ukrainian Large Black, there was a trend towards increasing in H_o values which might be a reflection of the fact that heterozygotic genotype was given preference during the selection process for this breed. The increase in Ukrainian Large Black heterozygote genotype was accompanied by a decrease in homozygote genotype, although these differences in genotypes distribution were not significant according to the Hardy-Weinberg equation. The PIC value for *CTSB* was also the highest in Ukrainian Large Black and Poltava Meat when compared to other breeds investigated. The average PIC is an ideal index to assess gene fragment polymorphisms. A PIC value over 0.5 indicates a highly polymorphic region, whilst PIC values in the range of 0.25-0.5 indicate a moderately polymorphic site, and a PIC value below 0.25 signifies a low polymorphic site (Hao et al., 2011). On the basis of the above, Ukrainian Large Black is the only breed with a level of heterozygosity sufficient for association studies.

The other polymorphism analysed in the exon 5 of the *CTSL* gene, was a synonymous mutation g.143C>T. Allele C was the predominant allele when compared to g.143T in all the breeds investigated with the lowest frequency (0.67) in Ukrainian Steppe White breed and the highest (0.98) in Myrgorod breed (Table 3). Breed-differences in the allele frequency were only statistically significant for Ukrainian Steppe Spotted when compared to Ukrainian Large White and Ukrainian Landrace. Similarly, allele T was the less frequent in Piétrain (MAF = 0.17) and Meishan (MAF = 0.10). The highest H_o and H_e were observed in Ukrainian Steppe Spotted followed by Piétrain. The level of heterozygosity in these breeds suggests that this gene could be used in future studies on associations with meat quality-related traits. In the other breeds studies, intermediate H_o and H_e values were observed in Ukrainian Steppe White, Poltava Meat and Ukrainian Large Black, and the lowest H_o and H_e were found in Myrgorod, Ukrainian Landrace and Ukrainian Large White.

In our study, H_o and H_e did not differ significantly in the breeds investigated and there was no deviation from the expected distribution of genotypes according to Hardy-Weinberg equation. These results suggest absence of

Table 3: Genotypes, allele frequencies and heterozygosity of the cathepsin L (*CTSL*) g.143C>T polymorphism in different breeds of pigs

	Genotype frequency			Allele frequency				
Breed (n)	**g.143CC**	**g.143CT**	**g.143TT**	**g.143C**	**g.143T**	**H_o[a]**	**H_e[b]**	**PIC[c]**
Ukrainian Large White (67)	0.88	0.12	0.00	0.94[e]	0.06	0.12	0.11	0.106
Poltava Meat (20)	0.75	0.25	0.00	0.88[def]	0.12	0.25	0.22	0.189
Ukrainian Landrace (35)	0.89	0.11	0.00	0.94[e]	0.06	0.11	0.11	0.106
Ukrainian Steppe Spotted (20)	0.45	0.45	0.10	0.67[d]	0.33	0.45	0.44	0.344
Ukrainian Steppe White (10)	0.70	0.30	0.00	0.85[def]	0.15	0.30	0.26	0.222
Myrgorod (20)	0.95	0.05	0.00	0.98[ef]	0.02	0.05	0.04	0.038
Ukrainian Large Black (42)	0.74	0.24	0.02	0.86[def]	0.14	0.24	0.24	0.212
Piétrain (9)	0.67	0.33	0.00	0.83[def]	0.17	0.33	0.29	0.242
Meishan (5)	0.80	0.20	0.00	0.90[def]	0.10	0.20	0.18	0.164

[a]Observed heterozygosity, [b]Expected heterozygosity, [c]Polymorphic information content,
Allele frequencies with different superscripts differ significantly,
$P < 0.05$ within Ukrainian pig breeds, (n) = number of animals

pressure of selection on the loci. The highest PIC for this locus was observed in Ukrainian Steppe Spotted. These findings are in a line with data of literature reporting g.143C as a dominant allele in Large White, Italian Landrace, Belgium Landrace, Piétrain, Meishan and Italian Duroc breeds with allele frequencies of 0.84, 0.85, 0.75, 0.90, 0.79 and 0.50, respectively (Fontanesi et al., 2010a; Dvorakova et al., 2011; Fontanesi et al., 2012). The H_o of this loci in Pietrain, Italian Landrace and Large White ranged from 0.18 to 0.27, and reached 0.33, 0.38 and 0.50 in Meishan, Belgium Landrace and Italian Duroc respectively (Fontanesi et al., 2010a; Fontanesi et al., 2012). Therefore, frequency of the minor allele, and respectively, heterozygosity of *CTSL* loci in Ukrainian Steppe Spotted breed were the highest not only when compared to the breeds investigated in the present study, but also when compared to data of the literature on other breeds. Ukrainian Steppe Spotted is a local Ukrainian breed, which is characterised by high backfat thickness, tender, tasty and juicy meat, a good ratio of tryptophan to oxyprolin, and it is adapted to the hot climate of Southern Ukraine (FAO, 2014). It is possible that the shift of allelic frequencies towards a higher frequency of the T allele in Ukrainian Steppe Spotted might have occurred during the process of selection for the mentioned above traits.

Data of the literature have reported a *CTSS* gene mutation in the 5' untranslated region associated with beneficial effects on average daily gain, lean content and backfat thickness (Fontanesi et al., 2010a). In the Ukrainian breeds and the two comparison breeds used in this study (Piétrain and Meishan), g.171G was the most frequent allele, with the allele frequency being in the range of 0.70 - 1.00 (Table 4). The g.171G allele frequency was significantly higher in the Ukrainian Large Black breed when compared to Poltava Meat, Ukrainian Landrace and Myrgorod with no differences between Poltava Meat, Ukrainian Landrace, Ukrainian Steppe Spotted, Ukrainian Steppe White and Myrgorod. The highest H_o (0.40 - 0.46) and the highest PIC values were observed in Meishan, Ukrainian Landrace and Myrgorod breeds. These data are consistent with finding of Fontanesi et al. (2010a) who demonstrated that g.171G was the predominant allele with a high frequency in Italian Large White, Italian Landrace, Italian Duroc, Pietran and Hampshire pigs, but not in Meishan with the level of heterozygosity being 0.08, 0.32, 0.41, 0.10, 0.28 and 0.00 respectively. Similarly to *CTSB* and *CTSL*, there were no deviations from expected distribution of genotypes according Hardy-Weinberg equation for the *CTSS* locus. Myrgorod breed had a high level of *CTSS* polymorphisms with H_o and H_e of 0.45 and 0.34 respectively which suggests that it might be a potential good candidate breed for associative studies.

The fourth caphepsin gene investigated in this study was *CTSK*. This gene is located in chromosome 4, directly upstream of the *CTSS* gene. However, the two mutations on *CTSK* and *CTSS* genes are only very moderately linked (D' = 0.40 and R^2 = 0.05). Therefore, the g.15G > A polymorphism of *CTSK* (see below) and the g.171G>A marker in *CTSS* can be studied independently.

In this study, research on *CTSK* gene was focused on g.15G>A SNP in intron 4. It was established that g.15G

Table 4: Genotypes, allele frequencies and heterozygosity of cathepsin S (*CTSS*) g.171G>A polymorphism in different breeds of pigs

	Genotype frequency			Allele frequency				
Breed (n)	**g.171GG**	**g.171GA**	**g.171AA**	**g.171G**	**g.171A**	**H_o[a]**	**H_e[b]**	**PIC[c]**
Ukrainian Large White (67)	1.00	0.00	0.00	1.00^{de}	0.00	0.00	0.00	0.000
Poltava Meat (20)	0.75	0.25	0.00	0.85^{e}	0.15	0.25	0.26	0.222
Ukrainian Landrace (35)	0.54	0.46	0.00	0.77^{e}	0.23	0.46	0.35	0.291
Ukrainian Steppe Spotted (20)	1.00	0.00	0.00	1.00^{de}	0.00	0.00	0.00	0.000
Ukrainian Steppe White (10)	0.80	0.20	0.00	0.90^{de}	0.10	0.20	0.18	0.164
Myrgorod (20)	0.55	0.45	0.00	0.78^{e}	0.22	0.45	0.34	0.284
Ukrainian Large Black (42)	0.90	0.10	0.00	0.95^{d}	0.05	0.10	0.09	0.091
Piétrain (9)	1.00	0.00	0.00	1.00^{de}	0.00	0.00	0.00	0.000
Meishan (5)	0.60	0.40	0.00	0.70^{de}	0.30	0.40	0.42	0.332

[a]Observed heterozygosity, [b]Expected heterozygosity, [c]Polymorphic information content,
Allele frequencies with different superscripts differ significantly
$P < 0.05$ within Ukrainian pig breeds, (n) = number of animals

Table 5: Genotypes, allele frequencies and heterozygosity of cathepsin K (*CTSK*) g.15G>A polymorphism in different breeds of pigs

	Genotype frequency			Allele frequency				
Breed (n)	**g.15GG**	**g.15GA**	**g.15AA**	**g.15G**	**g.15A**	**H_o[a]**	**H_e[b]**	**PIC[c]**
Ukrainian Large White (67)	0.97	0.03	0.00	0.99^{ed}	0.01	0.03	0.02	0.020
Poltava Meat (20)	0.90	0.05	0.05	0.93^{e}	0.07	0.05	0.13	0.122
Ukrainian Landrace (35)	1.00	0.00	0.00	1.00^{ed}	0.00	0.00	0.00	0.000
Ukrainian Steppe Spotted (20)	1.00	0.00	0.00	1.00^{ed}	0.00	0.00	0.00	0.000
Ukrainian Steppe White (10)	1.00	0.00	0.00	1.00^{ed}	0.00	0.00	0.00	0.000
Myrgorod (20)	0.60	0.35	0.05	0.78^{d}	0.22	0.35	0.34	0.284
Ukrainian Large Black (42)	1.00	0.00	0.00	1.00^{ed}	0.00	0.00	0.00	0.000
Piétrain (9)	1.00	0.00	0.00	1.00^{ed}	0.00	0.00	0.00	0.000
Meishan (5)	0.40	0.40	0.20	0.60^{ed}	0.40	0.40	0.48	0.365

[a]Observed heterozygosity, [b]Expected heterozygosity, [c]Polymorphic information content
Allele frequencies with different superscripts differ significantly
$P < 0.05$ within Ukrainian pig breeds, (n) = number of animals

was the prevailing allele with frequency values ranging from 0.60 to 1.00 in all the breeds analysed (Table 5). The allele G was fixed in the Ukrainian Landrace, Ukrainian Steppe Spotted, Ukrainian Steppe White, Ukrainian Large Black and Piétrain breeds. The highest frequency of the minor g.15A allele was observed in Meishan

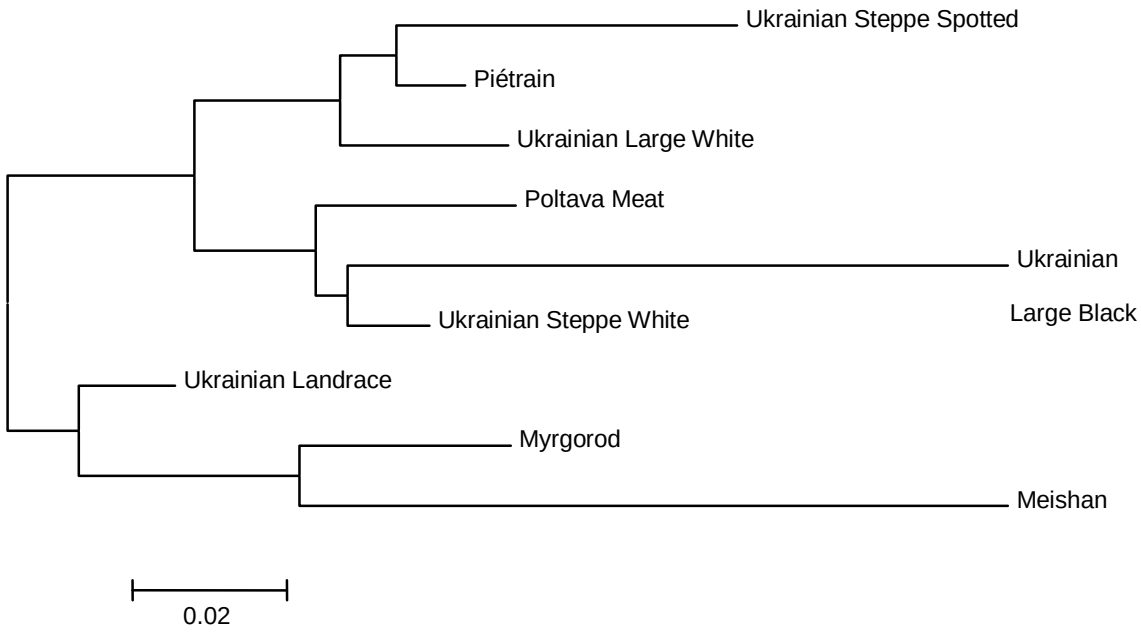

Figure 1: Dendogram representing genetic distances (neighbor-joining method) between the nine pig breeds constructed from the cathepsin genes variability data.

(MAF = 0.40) and Myrgorod breeds (MAF = 0.22). A significant difference in the allele frequency was established only between Poltava Meat and Myrgorod. These results are consistent with a report by Fontanesi et al. (2010b) on Italian Large White, Italian Duroc, Italian and Belgian Landrace, Pietrain pigs where a higher frequency of g.15G compared to g.15A allele was observed. In all the breeds investigated, with the exception of Poltava Meat, there were no significant differences between H_o and H_e. In Poltava Meat, the H_o value was almost 3 times lower than H_e (χ^2 = 13.3). Consequently, for this breed, we found statistical confirmation (χ^2 = 18.9) of variation in distribution in *CTSK* genotypes from expected distribution. This suggests that Poltava Meat breed has undergone a strong pressure of selection in this locus during the breeding for reduced backfat thickness and increased weight of lean cuts which might have influenced the balance of *CTSK* genotypes. The specific role of *CTSK* in muscle physiology and/or meat maturation is unknown, however there are reports indicating that this protease participates in cardiac muscle regeneration after ischemic injuries (Vassalle and Iervasi, 2013). Therefore, it is possible that this protein plays a similar role in skeletal muscle. To the best of our knowledge, there is only one paper which considers *CTSK* as a candidate gene for fat accumulation, backfat thickness and average weight gain (Fontanesi et al., 2010b). Similarly to *CTSS* locus, a relatively high level of polymorphisms in *CTSK* was observed only for Myrgorod breed (H_o = 0.35, H_e = 0.34). Therefore, this local Ukrainian breed, which originated from Black Spotted pig breed in Poltava region and which is known for its tender, juicy and tasty meat with high intramuscular fat content, is attractive for undertaking association studies not only on *CTSS* but also on *CTSK* locus.

Data on sequence variability for the four cathepsin genes were used to represent, by means of a dendogram, the genetic relationship between the nine breeds analysed (Figure 1). The breeds were grouped in three main clades. The first clade included the lean Piétrain breed, Ukrainian Large White and Ukrainian Steppe Spotted breeds. The Piétrain breed has been used in Ukrainian breeding programs for increasing their lean content which has influenced Ukrainian breeds genotypes and alleles frequencies. The second clade groups three Ukrainian breeds (Poltava Meat, Ukrainian Large Black and Ukrainian Steppe White).

These three breeds are considered to be “meat-and-fat” type pigs which mean that they have a higher subcutaneous and intramuscular fat content whilst maintaining excellent meat quality characteristics. Finally, the Myrgorod and Meishan breeds have been combined in the third clade. These two breeds have a much higher fat content compared to other breeds investigated. In particular, Myrgorod is known for its excellent meat flavour due to a high content of intra- and inter-muscular fat. Despite of the general low level of polymorphisms found, the genotypic information on the cathepsin genes allowed to capture differences in meat and fat aptitudes of the Ukrainian, European and Chinese breeds. This information can be used for designing further studies on involvement of these genes in pigs fattening and meat tenderisation.

CONCLUSIONS

To summarise, this is the first study which investigates cathepsin polymorphisms in Ukrainian pig breeds. The study established breed-specific differences in the allele frequencies and informativity of polymorphisms in the *CTSB, CTSL*, *CTSS* and *CTSK* genes. The following are of particular interest: (i) locus *CTSB* in Ukrainian Large Black breed; (ii) locus *CTSL* in Ukrainian Steppe Spotted, and (iii) loci *CTSS* and *CTSK* in Myrgorod breed. The segregation of SNPs in meat quality candidate genes with intermediate or high polymorphism levels in the Ukrainian breeds Myrgorod, Ukrainian Steppe Spotted and Ukrainian Large Black, are of interest for future evaluation in association studies with the view of complementing selection indexes with molecular information.

AKNOWLEDGEMENTS

This research was supported by a research grant from the National Academy of Agricultural Sciences, Ukraine (No DP 0111U004024), EU Marie Curie International Research Staff Exchange Grant “Excelmeat” (No 246760) and Biotechnology and Biological Sciences Research Council Pathfinder grant (No BB/FOF/PF/5/11).

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